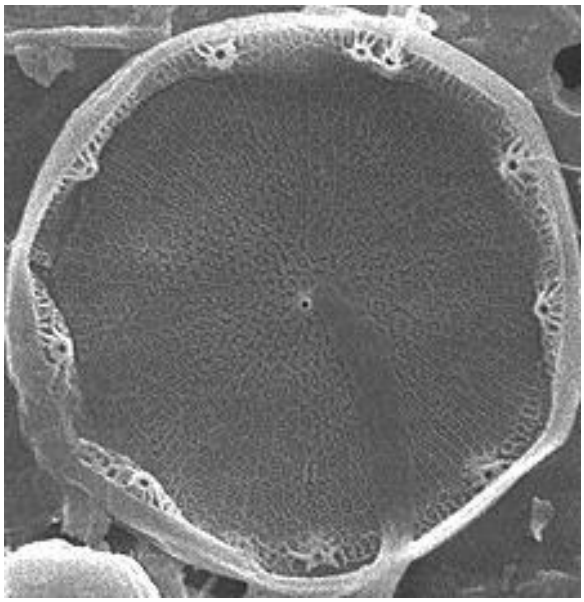
Photoacclimation and nitrogen limitation effects on the biomass growth and use of D1 protein repair cycle during photoinhibition in *Thalassiosira oceanica* (bacillariophyceae) and *Tetraselmis sp.* (chlorophyceae).



Thalassiosira oceanica
[Hassle 1983]

Tetraselmis sp.
[Provasoli-Guillard National Center for Marine Algae and Microbiota]

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Abstract

The influence of photoacclimation and nitrogen availability on the growth and the photoinhibitory response (D1 protein repair cycle) of two phytoplankton species, *Thalassiosira oceanica* (bacillariophyceae) and *Tetraselmis sp.* (chlorophyceae) was assessed using a pulse amplitude modulated fluorometer. Cultures were grown under three different irradiances (9, 50, and 125 μmol photon m^{-2} s^{-1}). The experiments were performed when the algae were nutrient replete and nitrogen limited. In the first experiment, the algae were exposed to a range of irradiances for 30 min. and the photosynthetic efficiency of PS II *(*Fv/Fm) was measured. In the second experiment, the algae were exposed to a high irradiance (950 μmol photon m^{-2} s^{-1}) for 15 min. and the Fv/Fm recovery was followed. Lincomycin, a chloroplast-encoded protein inhibitor, was used to estimate the role of D1 protein repair cycle during photoinhibition. The data suggest that increasing growth irradiance has a positive effect on the growth of *T. oceanica* and *Tetraselmis*, while nitrogen limitation stops their growth. The D1 protein repair cycle appears to be more active in *T. oceanica* during recovery from high irradiance when compared to *Tetraselmis.* The data also suggests that the algae's photoacclimation state and nitrogen availability can have an effect on the algae's photosynthetic efficiency under increasing irradiance. However, it is unclear to what extent the D1 protein repair or the xanthophyll cycle is responsible.

INTRODUCTION

The ocean is a dynamic environment in which phytoplankton must deal with rapid changes in resource concentrations, particularly irradiance [MacIntyre 2000, Schubert 2001]. Incident irradiance experienced by algae is determined by vertical mixing in the water column, seasons, diurnal cycles, weather, and optical properties of particles, detritus, and sediment [Schubert 2001, Kirk 1994]. Low irradiance can lead to a decrease in algae growth, but with no immediate risk of cellular death. Rapid and large increases in irradiance can lead to a decrease in photosynthetic efficiency and cellular viability [Six 2007].

Algae have several photoprotective mechanisms to deal with high irradiance, so they can optimize growth and minimize photodamage. One of which is the enzymatic conversion of xanthophylls (the xanthophyll cycle), which has been recognized as an important regulatory process that can minimize photodamage to photosystem II (PSII). This process involves the thermal dissipation of harmful excess energy away from PSII [Demming-Adams 1992]. There are two main xanthophyll cycles for algae, the violaxanthin–antheraxanthin–zeaxanthin (VAZ) and the diadinoxanthin–diatoxanthin (Dd-Dt) cycle [van de Poll *et al.* 2010]. The VAZ cycle is mainly used by green algae (chlorophyceae), while Dd-Dt is the main cycle used by diatoms (bacillariophyceae) [Goss *et al.* 2010]. These cycles differ with regard to the enzymatic de-epoxidation and epoxidation of the xanthophylls [Wilhelm *et al.* 2006]. For example, de-epoxidation of the VAZ cycle cannot be maintained in darkness, while this can occur with the Dd-Dt cycle [Goss *et al.* 2010]. Also, the need for efficient xanthophyll cycle dependent photoprotection is less important in green algae than in diatoms, where xanthophylls represent the major photoprotection mechanism. Green algae have other unique mechanisms for photoprotection and minimizing photodamage, e.g., state transitions [Goss *et al.* 2010, van de Poll *et al.* 2010].

If photoprotective mechanisms fail to neutralize excess irradiance, then the overexcitation of PSII leads to the production of reactive oxygen species (ROS), which damage the photosynthetic apparatus [Muller *et al*. 2006]. The reaction center protein D1 is the component of PSII most prone to photooxidative damage. D1 binds the primary donors and acceptors active in PSII electron transport [Mattoo *et al.* 1984]. Algae can counter the inactivation of PSII, with repair, through proteolytic removal of photodamaged D1 protein and the coordinated insertion of newly synthesized D1 into the thylakoid membrane [Park 1995, Tyystjarvi 1996, Shelly 2002, Nixon 2005, Nishiyama 2006]. This is a metabolically expensive process [Aro *et al.* 2004]. D1 protein has the fastest turnover of all PSII components [Bouchard 2006]. Photoinhibition through D1 protein degradation can occur starting at irradiances as low as 5 μmol photon m^{-2} s^{-1} [Edelman 2008, Jansen 1999]. Protein synthesis is dependent on the availability of inorganic nitrogen. Therefore, limited nitrogen concentrations may also lead to a decrease in photosynthetic efficiency [Kolber 1988].

Light harvesting and excessive energy dissipation capacity can be adjusted on a timescale of hours to days through the process of *de novo* pigment synthesis [Kana *et al.* 1997]. As a result, photosynthesis and photoprotection characteristics

are strongly influenced by the radiation history of the algae. Studies have shown that high light acclimated cultures have a faster de-epoxidation of xanthophyll cycle pigments after high light exposure, and a greater D1 repair cycle activity when compared to low light acclimated cells exposed to high light [Falkowski and LaRoche 1991, Lavaud 2002, van de Poll *et al.* 2005, 2006, 2011]. Phytoplankton's ability to cope with different resource concentrations can vary greatly between species, which in turn can influence the community structure and biomass [Tilman 1982].

In this paper, fluorescence parameters were used to investigate the effect of photoacclimation & nitrogen limitation on the biomass growth & photoinhibitory response of *Thalassiosira oceanica* and *Tetraselmis sp.*, with a larger focus on the D1 protein repair cycle. It was hypothesized that larger growth irradiances would cause the algae to grow faster, while nitrogen limitation would stop the algae growth. Furthermore, it was hypothesized that the use of the D1 protein cycle under increasing irradiance is influenced by the algae's photoacclimation state, species type, and nitrogen availabilty. It must be taken into account, that the variability of photoprotective mechanisms (e.g. xanthophyll cycle) under different photoacclimation states and algae species can also have an effect on the use of the D1 repair cycle.

MATERIALS AND METHODS

Cultivation

Thalassiosira oceanica (bacillariophyceae) (strain CCMP 1616, Mediterranean Sea) and *Tetraselmis sp.* (chlorophyceae) (strain RCC 233, Mediterranean Sea) were used for the experiments. Both algae have a similar cell size of ~ 7 µm. Four lab culture series of each algae were grown in a sterile 200 mL Erlenmeyer flask containing filtered (0.2 µm) low nitrogen (N:P ratio = 2:1) altered F/2 enriched seawater [Guillard and Ryther 1962, Guillard 1975]. 150 µmol N & 75 µmol P were added to the F/2 medium instead of 882 µmol N & 36.2 µmol P from the standard F2 medium. The low nitrogen concentration allowed the algae to reach nitrogen limited instead of a phosphorus limited state. Two cultures in each series were used for experimentation when the algae were nutrient replete (middle of exponential growth phase), and two different cultures were used when the algae were nitrogen limited (2 days of growth rate = 0). The cultures were grown under three irradiances (9, 50, and 125 µmol photon m^{-2} s^{-1}). The Biolux (Osram) fluorescent lamps had a light-dark cycle of 12h-12h and the waterbath was maintained at a temperature of 20°C. This supports high growth rates for both species and approximate summer temperatures for temperate/subtropical- waters.

Measurements

1) Chlorophyll-*a* fluorescence

A Walz pulse-amplitude modulated (PAM) fluorometer was used to determine the theoretical maximum light utilization efficiency of PS II (Fv/Fm, the ratio of variable fluorescence Fv to maximum fluorescence Fm, formula 1) and minimum fluorescence (F_0, determined using PAM's non-photochemistry-inducing light). Chlorphyll-a fluorescence is used to estimate the relative number of active PS II (Consalvey 2005). The PAM fluorometer was blanked with filtered (0.2 μm) seawater before each analysis. All measurements were performed in duplicate (i.e., algae were obtained from two separate cultures).

$$F_v/F_m = \frac{F_m - F_o}{F_m} \qquad \text{Formula (1)}$$

2) Irradiance

Irradiance was measured in air, with a QSL-100 (Bio-spherical Instruments) with a spherical sensor. The sensor was held just above the water's surface.

Treatments

1) Growth Irradiance (GI)

The three irradiances used during algae growth will be indicated in this paper as GI 9, GI 50, and GI 125.

2) Protein inhibitor

Lincomycin, an inhibitor which stops the synthesis of chloroplast encoded proteins, was used to estimate the role of de novo D1 protein synthesis during photoinhibition [Bouchard 2005]. There were two inhibitor treatments used during this research. One was with no addition of lincomycin (L-) to the algae samples, and the other was with the addition of $0.6x10^{-3}$ mol^{-1} (final concentration) of lincomycin (L+) (stock solution prepared in 96% ethanol). The treated algae samples were kept in the dark at 20°C for 15 minutes of acclimation (DA) before the PSII efficiency vs. irradiance, and

photoinhibition-recovery experiments (both experiments described later).

3) Nitrogen Availability

The experiments were performed when the algae were nutrient replete (E, exponential growth phase) and nitrogen limited (S, stationary growth phase). To follow growth, daily F_0 measurements were done using the same PM gain & OUT gain in the PAM settings between each day (fig. 1). The algae samples were diluted with filtered seawater whenever the F_0 measurements exceeded the PAM F_0 range. F_0 was then calculated using the diluted F_0 value. For example, if the algae sample was diluted by a factor of 10, then the F_0 measurements obtained were multiplied by 10 in order to obtain the true F_0 value. Daily Fv/Fm measurements were done with F_0 values between 300 and 500 during the algae growth. The measurements were done quickly (< 5 min.) in order to avoid recovery of the PSII system under low light conditions.

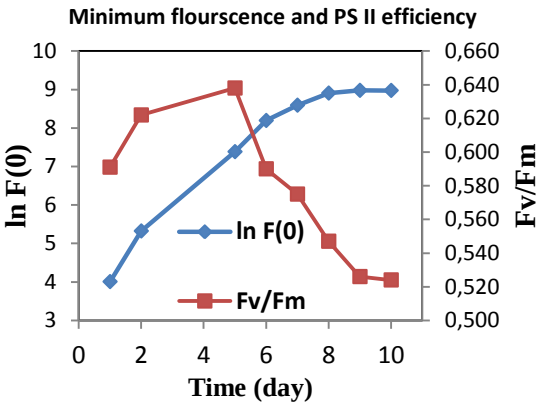

Fig. 1. Example Biomass ln(F_0) and PSII effficiency (Fv/Fm) dynamics during algae growth. Example taken from *T. oceanica* grown in GI 50.

Biomass & F_0

Biomass growth was estimated by following the change in the algae's minimum fluorescence (F_0). A correlation between algae biomass and F_0 is required in order to use F_0 as an estimate for biomass growth. To determine if there is a correlation between biomass and F_0 for the experimental algae, the cell count of *T. oceanica* and *Tetraselmis* was obtained using a Sedgewick-Rafter counting chamber. This was done daily, till the start of the nitrogen limited phase, using 1 mL algae samples. The algae samples were fixed using Lugol's iodine solution. The cell count was then compared to the daily F_0 (PAM) measurements. The algae used for biomass vs. F_0 were grown under an irradiance of 50 µmol photon m^{-2} s^{-1}. Two cultures were grown for *T. oceanica* and two cultures were grown for *Tetraselmis.*

PS II efficiency vs. irradiance experiment

The photoinhibitory response was investigated by exposing the algae to an array of irradiances and measuring the change in the photosynthetic efficiency of PS II (Fv/Fm). Glass vials (10 mL) were filled with 2.5 mL of algae and were placed (after DA) into slots seen in figure 2. The light in these slots was modulated by neutral density filters, creating 10 light levels. (table 1). One algae vial was wrapped in aluminum foil for dark control. The slots and dark control vials were placed in a water bath (20°C) which was lit from the bottom by a Phillips MHN-TD 250W lamp. Fv/Fm was measured and the % DA Fv/Fm was calculated for each algae series after being exposed to different irradiances for 30 minutes (fig. 3). This was done separately for both algae, with all three growth irradiances, during exponential and stationary growth, and with or without the addition of the inhibitor.

Table 1. Irradiance (µmol photon m^{-2} s^{-1}) used during PS II efficiency experiment.

17	32	70	82	132
176	260	345	493	1007

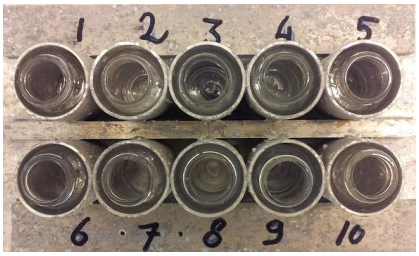

Fig. 2. Slots that let in different amounts of irradiance for use during PS II efficiency experiment.

Photoinhibition-recovery experiment

The photoinhibitory response was further investigated by following the Fv/Fm recovery after excess irradiance exposure. Quartz vials (100 mL) were filled with 20 mL of algae and were placed (after DA) into the water bath described in the previous paragraph. The algae were exposed to an irradiance of 950 µmol photon m^{-2} s^{-1} (HL) for 15 minutes. The light was then shut off and the algae were left in the dark water bath to recover. Fv/Fm was measured using 2 mL of the algae and then recalculated into % DA Fv/Fm. Measurements were

done after DA, after HL exposure, and in 20 min. intervals for 2 hours (fig. 4). This was done separately for both algae, with all three growth irradiances, during exponential and stationary growth, and with or without the addition of the inhibitor.

Differences in algae species, light, and nutrient conditions can result in different base values for Fv/Fm. The Fv/Fm for the PSII efficiency vs. irradiance experiment and the photoinhibition-recovery experiment will be expressed in this paper as a % of dark acclimated (DA) Fv/Fm in order to allow the comparison of light responses (fig. 3 & 4).

Statistics

Correlations were estimated using a pearson product-moment correlation coefficient. Due to a low sample size (n=2), no true statements can be made on significance. A correlation was accepted at $r^2 = 0.976$(crit. value for n=3, $\alpha = 0.1$).

Differences between groups were tested for significance with a type 1 single factor analysis of variance (ANOVA) and were considered significant at $p<0.05$. All ANOVA tests were done with n=2. Sample size will be given if $n \neq 2$.

RESULTS

Biomass vs. F_0

There was a positive correlation between daily ln cell count and ln F_0 measurements for *T. oceanica* and *Tetraselmis sp.* (fig. 5), (Pearson: $r^2=0.984$, $r^2=0.992$).

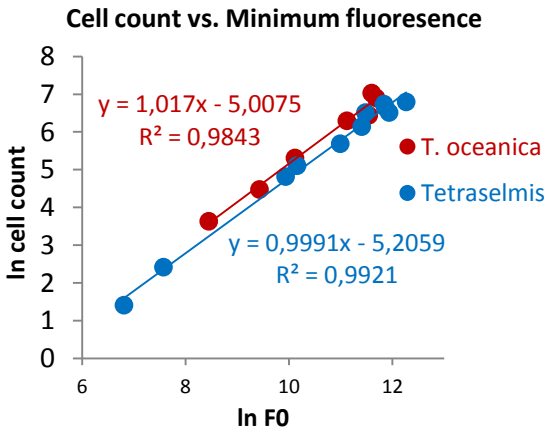

Fig. 5. ln cell count plotted against ln F_0 for *T. oceanica*(red) and *Tetraselmis* (blue) (averages plotted, n=2)

Biomass & Fv/Fm during growth

Higher growth irradiances (GI) had a positive effect on the growth rate of *T. oceanica* and *Tetraselmis* (table 2). *T. oceanica* had higher growth rates and entered a nitrogen limited faster compared to *Tetraselmis* for all GI. It took both algae in the GI 9 cultures significantly longer to enter the nitrogen limited state compared to GI 50 and GI 125. Nitrogen limitation stopped the growth of *T. oceanica* and *Tetraselmis*.

When comparing growth phases, only the GI 50 and GI 125 cultures for *T. oceanica* and *Tetraselmis* had significant differences in Fv/Fm. The stationary growth phase (nitrogen limited) had a lower Fv/Fm. No significant differences were observed in Fv/Fm between growth phases in the GI 9 cultures.

When comparing growth irradiances, the GI 125 cultures showed a significant decrease in Fv/Fm during the nutrient rich phase and the nitrogen limited phase when compared to GI 9 cultures. This was observed for *T. oceanica* and *Tetraselmis*. The GI 50 cultures had a significant decrease in Fv/Fm only in the nitrogen limited phase when compared to GI 9 cultures. The nutrient rich phase GI 50 cultures for both algae did not have a significant difference in Fv/Fm when compared to the GI 9 cultures.

PS II efficiency vs. irradiance experiment

All treatments had a mean maximum % DA Fv/Fm of 94% ± 9% between 32 & 70 µmol photon m^{-2} s^{-1} (n=96) (fig. 3). *T. oceanica* GI 125 had the highest maximum % DA Fv/Fm of 118%.

For all irradiances > 82 µmol photon m^{-2} s^{-1}, GI 9 and GI 50 *T. oceanica* and *Tetraselmis* had a significant decrease in % DA Fv/Fm in the inhibitor treatment compared to the non-inhibitor treatment. Significant differences between inhibitor treatments could be seen for GI 125 nutrient replete *Tetraselmis* starting at a low irradiance of 17 µmol photon m^{-2} s^{-1}. For GI 125 *T. oceanica*, significant differences occured at 176 µmol photon m^{-2} s^{-1}.

The largest differences in % DA Fv/Fm between the non-inhibitor and inhibitor treatments were observed in *T. oceanica*.

In the non-inhibitor treatment, the % DA FV/Fm was higher than 50% for all data points with irradiance < 493 µmol photon m^{-2} s^{-1}. In the inhibitor treatment, the % DA Fv/Fm for most cultures dropped below 50% between irradiance 132 and 260 µmol photon m^{-2} s^{-1}.

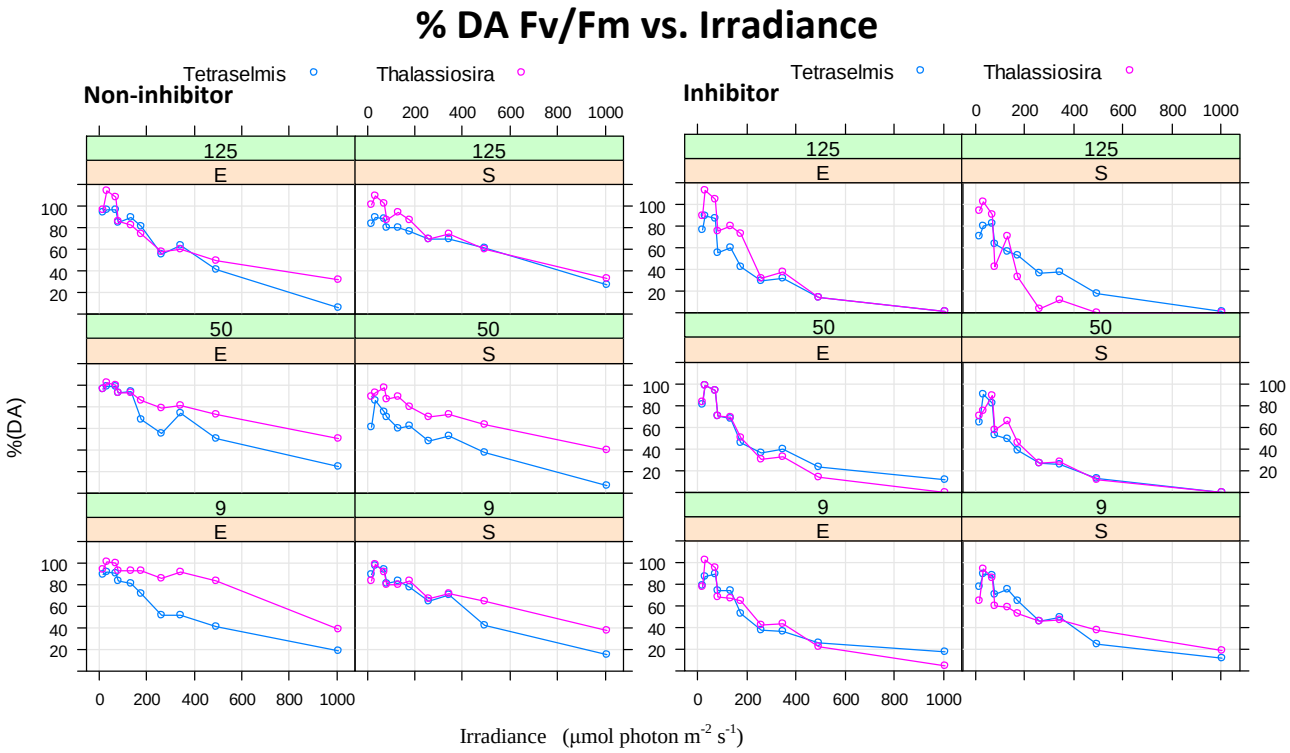

Fig. 3. Mean values (n=2) % DA Fv/Fm plotted against irradiance with non-inhibitor treatment (left graphs) and inhibitor treatment (right graphs) for *Tetraselmis* and *T. oceanica*. Growth irradiance (9, 50, 125 µmol photon m^{-2} s˙1) and nutrient availability (E = nutrient replete, S = nitrogen limited) are indicated above each graph.

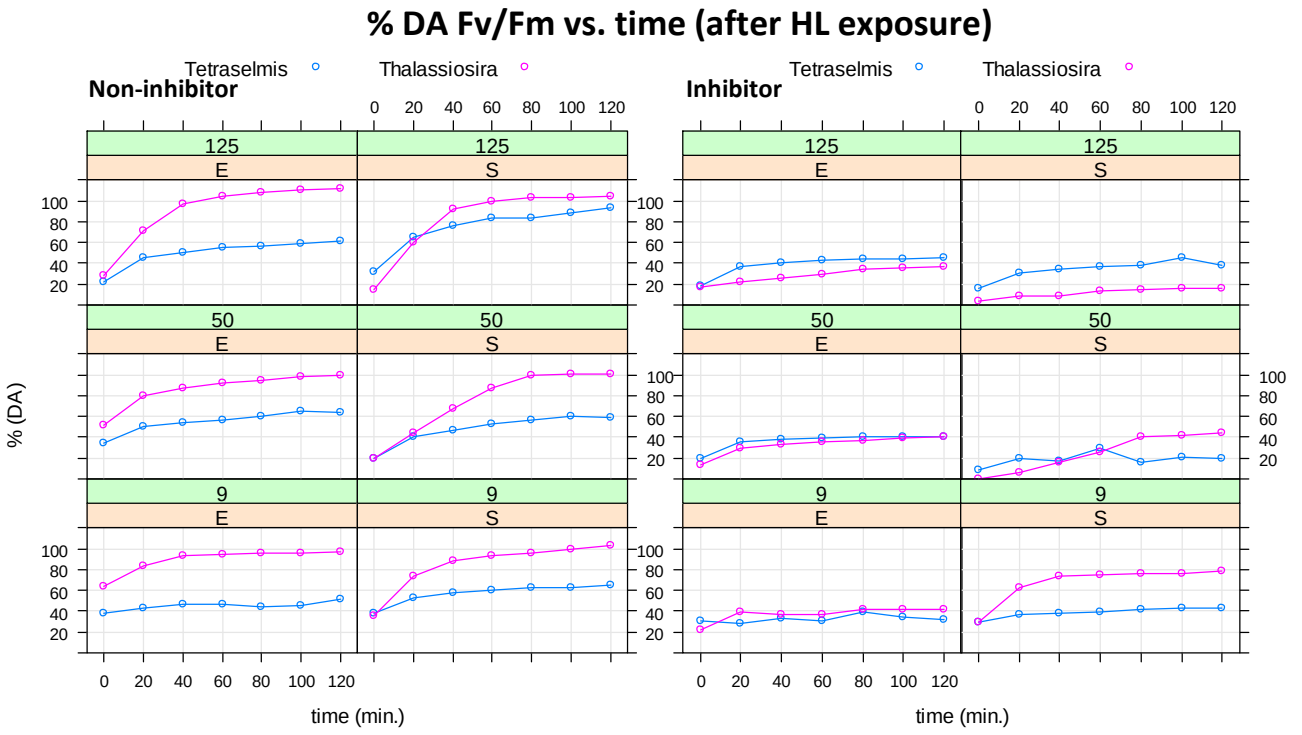

Fig. 4. Mean values (n=2) % DA Fv/Fm in 20 minutes intervals during dark recovery after HL (950 µmol photon m^{-2} s^{-1}) exposure with non-inhibitor treatment (left graphs) and inhibitor treatment (right graphs) for *Tetraselmis* (red) and *T. oceanica* (blue). Growth irradiance (9, 50, 125 µmol photon m^{-2} s˙1) and nutrient availability (E = nutrient replete, S = nitrogen limited) are indicated above each graph.

Table 2. Mean and standard deviation (n=2) of growth rate (GR, unit = ln F_0) and PS II efficiency (Fv/Fm) for *Tetraselmis* and *T. oceanica*. Growth Irradiance (GI) and Day of experiment (Day) are given in table. Samples were not dark acclimated.

		Nutrient replete			Nitrogen limited		
Species	GI	Day	GR	Fv/Fm	Day	GR	Fv/Fm
Tetraselmis	9	15	0,1±0,0	0,686±0,01	67	0,0±0,0	0,697±0,00
	50	6	0,6±0,0	0,702±0,00	23	0,0±0,0	0,413±0,01
	125	4	0,9±0,1	0,632±0.01	19	0,1±0,0	0,493±0,02
T. Oceanica	9	13	0,3±0,0	0,661±0,01	40	0,0±0,0	0,665±0,02
	50	5	0,8±0,0	0,628±0,01	9	0,1±0,0	0,517±0.01
	125	4	1,6±0,0	0.485±0,02	8	0,0±0,0	0,337±0,01

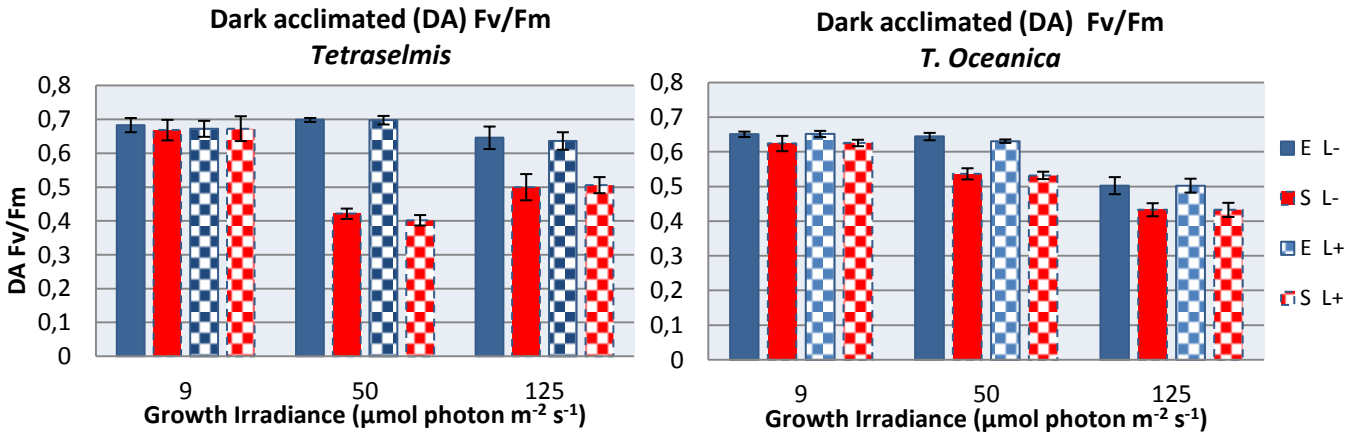

Fig. 6. Pooled mean and standard deviation dark acclimated (DA) Fv/Fm (n=4) for PSII efficiency vs. irradiance experiment and photoinhibition-recovery experiment. *Tetraselmis* graph (left) and *T. oceanica* graph (right). Samples were dark acclimated (15 min.). Legend key: nutrient replete (E), nitrogen limited (S), non-inhibitor treatment (L-), inhibitor treatment (L+).

Nutrient rich *T. oceanica* in the non-inhibitor treatment, grown in GI 9 and GI 50, had a higher % DA Fv/Fm than *Tetraselmis* for all irradiance greater than 82 and 176 µmol photon m^{-2} s^{-1}, respectively.

Nitrogen limited *T. oceanica* (all GI) in the inhibitor treatment had significantly lower % DA Fv/Fm at irradiance > 82 µmol photon m^{-2} s^{-1} compared to the same irradiances in the non-inhibitor treatment. Nitrogen limited *Tetraselmis* 9S had lower % DA Fv/Fm at irradiance > 176 µmol photon m^{-2} s^{-1}, *Tetraselmis* 50S irradiance > 260 µmol photon m^{-2} s^{-1}, and *Tetraselmis* 125S at irradiance > 82 µmol photon m^{-2} s^{-1}.

For *T.* oceanica in the inhibitor treatment at the maximum irradiance of 1007 µmol photon m^{-2} s^{-1}, all cultures except for 9S reached a % DA Fv/Fm of 0%. The 125S *T. oceanica* cultures reached 0% at 260 µmol photon $m^{-2}s^{-1}$. *Tetraselmis* 125S remained above 0% till 493 µmol photon m^{-2} s^{-1}. *Tetraselmis*, 9E, 9S, and 50E cultures did not reach 0%. None of the algal cultures reached an Fv/Fm of 0% in the non-inhibitor treatment.

Photoinhibition-recovery experiment

All *T. oceanica* in the non-inhibitor treatment reached 100% DA Fv/Fm recovery within 2 hours. *Tetraselmis* recovered on average 60% of its DA Fv/Fm (fig. 4). Both algae in the inhibitor treatment recovered on avergage 40% of their % DA Fv/Fm. *T. oceanica* and *Tetraselmis* had a significant decrease in % DA Fv/Fm in the inhibitor treatment compared to the non-inhibitor treatment for each time interval. The largest differences in % DA Fv/Fm recovery after 2 hours between the non-inhibitor and inhibitor treatments were observed in *T. oceanica.*

For all non-inhibitor treatments, most % DA Fv/Fm recovery occurred within 40 minutes after stopping the high light exposure. This initial recovery was much higher in *T. oceanica* compared to *Tetraselmis.* The initial % DA Fv/Fm recovery in *T.*

oceanica was also greater with increasing growth irradiances. All *T. oceanica* nitrogen limited cultures in the non-inhibitor treatment had a much greater initial recovery when compared to the nutrient replete cultures. There was no % DA Fv/Fm initial recovery observed in the inhibitor treatments, (except *T. oceanica* 9S).

Discussion

The effect of photoacclimation and nitrogen limitation on the biomass growth & photoinhibitory response of *T. oceanica* (bacillariophyceae) and *Tetraselmis* (chlorophyceae) was investigated, with a focus on the D1 protein synthesis.

Biomass vs. F_0

There was a positive correlation between the algae's minimum fluorescence and daily cell counts, which means that a PAM's minimum fluorescence can be used as an indicator for phytoplankton biomass growth in the experiment performed in this paper. Correlation results between biomass and F_0 are in line with the findings from other studies [Consalvey 2005, Honeywill 2002]. By using minimum fluorescence, biomass growth can be assessed much faster than by manually counting the algae. Even though minimum fluorescence can be used to estimate biomass growth, no exact statements can be made about the actual number of cells in a sample. A PAM's minimum fluorescence is a measure of the amount of chlorophyll-a in the sample. Cellular chlorophyll-*a* varies with algal species and growth conditions, e.g. light and nutrients. Therefore, minimum fluorescence cannot be used to compare biomass between different algae.

Algae growth & nitrogen limitation

With sufficient nutrients, higher growth irradiances have a positive effect on the biomass growth rate of *T. oceanica* and *Tetraselmis*. For both algae, the largest increase in biomass per day was in GI 125. Nitrogen limitation stopped the biomass increase of the algae, although this was unclear for GI 9 due to low growth rates already present under adequate nutrient concentrations (table 2). Irradiance might be the factor limiting algae growth in the GI 9 samples. Also, there was no significant decrease in Fv/Fm in the nitrogen limited GI 9 cultures. Nitrogen limitation did have a negative effect on the Fv/Fm of the GI 50 and GI 125 cultures for *T. oceanica* and *Tetraselmis*. Nitrogen concentrations seem to play a more crucial in algae growing under higher irradiances, and less so under low irradiances. It could be that the effect of low nitrogen concentrations during algae growth is enhanced under high irradiances due to a decrease in chlorophyll-*a* and increase in protein concentrations [Kolber 1988].

Dark acclimation & lincomycin

No differences were observed between non-inhibitor (L-) and inhibitor (L+) treatments after dark acclimation for *T. oceanica* and *Tetraselmis* (all GI, fig. 6). This was true for *T. oceanica* and *Tetraselmis* in the exponential and stationary growth phase. It can be concluded that lincomycin does not affect the Fv/Fm of these algae when they are in the dark. The effect of lincomycin on the algae's Fv/Fm was seen only after photodamage to the D1 reaction center has taken place during the PSII efficiency vs. irradiance experiment and photoinhibition-recovery experiment.

Recovery of the photosynthetic efficiency was observed in dark acclimated stationary GI 125 *T. oceanica* cultures. The recovery is seen when comparing the stationary GI 125 *T. oceanica* DA Fv/Fm (fig. 6) with the daily Fv/Fm measurements for the same culture (table 2). Some recovery was observed in the other cultures, but this increase in Fv/Fm was not significant. Recovery of the photosynthetic efficiency could be possible through the xanthophyll cycle. However, there was only recovery in the stationary GI 125 *T. oceanica* cultures. It could be that there were procedural errors committed while obtaining the DA Fv/Fm for the GI 125 *T. oceanica* cultures.

Previous studies have shown that lincomycin has no effect on the concentrations of xanthophylls during shifts from low to high irradiance. However, the presence of lincomycin appears to negatively influence the epoxidation rate of xanthophylls when a shift from high to low irradiance occurs. The slower rate of xanthophyll epoxidation could be attributed to the presence of damaged D1 complexes, where de-epoxidized xanthophylls are needed to avoid further damage to the PSII apparatus [Domingues 2012, Bachmann 2004, Wu 2012, Jin 2003].

PS II efficiency vs. irradiance experiment

T. oceanica and *Tetraselmis* had a maximum relative PSII efficiency between irradiances 32 &

70 μmol photon m^{-2} s^{-1}, regardless of growth irradiance, nutrient concentrations, or inactivation of D1 protein synthesis as observed during the PSII efficiency vs. irradiance experiment (fig. 3). In this light range, photoinhibition seems to be minimal, while photosynthetic light utilization is at a maximum. GI 125 *T.* oceanica had a % DA Fv/Fm larger than 100% in the 32 - 70 μmol photon m^{-2} s^{-1} light range. This shows that recovery of PS II in GI 125 *T. oceanica* samples is still taking place at those light levels. The recovery is most likely due to the xanthophyll cycle, for the reason that there were no differences in relative photosynthetic efficiency between the inhibitor treatments in those *T. oceanica* samples.

The synthesis of D1 protein starts playing a significant role in GI 9 and GI 50 *T. oceanica* and *Tetraselmis* for irradiances > 82 μmol photon m^{-2} s^{-1}. For these light levels, differences in % DA Fv/Fm between the non-inhibitor and inhibitor treatment are significant for both algae, during nutrient replete and nitrogen limited phase. It is clear that the photoprotective mechanisms (e.g. xanthophyll cycle) cannot negate photodamage to PS II at these light levels and that repair of the D1 reaction centers is required. Nutrient replete GI 125 *Tetraselmis* had a significant decrease in % DA Fv/Fm between inhibitor treatments starting at a lower 17 μmol photon m^{-2} s^{-1}. This suggests that the irradiance levels during cultivation in GI 125 were enough to cause photodamage to *Tetraselmis'* PS II reaction centers and that the D1 protein repair cycle is already active. Nutrient replete GI 125 *T. oceanica* had a significant decrease in % DA Fv/Fm between inhibitor treatments starting at a greater 176 μmol photon m^{-2} s^{-1}. Nutrient replete GI 125 *T. oceanica* also had a lower DA Fv/Fm than GI 9 and GI 50, and therefore had fewer active photosystems. It seems that the nutrient replete GI 125 *T. oceanica*'s fewer active photosystems could be protected by photoprotective mechanisms (e.g. xanthophyll cycle) from photodamage under higher irradiance when compared to the *T. oceanica* GI 9 and GI 50 cultures. Furthermore, the GI 9 *T. oceanica* had a greater decrease in % DA Fv/Fm between inhibitor treatments when compared to the higher GI's. This shows that there could be photodamage occurring in the GI 9 samples, which indicates an increased dependence on the activity of the D1 protein repair cycle. It is possible that the large decrease in % DA Fv/Fm between inhibitor treatments in GI 9 *T. oceanica* can be attributed to a greater DA Fv/Fm than GI 50 and GI 125. This could mean that there are more active photosystems present in GI 9 that need to be repaired once photodamage occurs.

T. oceanica had a greater decrease in % DA Fv/Fm between inhibitor treatments when compared to *Tetraselmis.* This is an indication that the D1 protein repair cycle is more important in *T. oceanica*.

There were no clear trends in the % DA Fv/Fm difference between the nutrient replete (E) and nitrogen limited (S) phases for *T. oceanica* and *Tetraselmis* in the PSII efficiency vs. irradiance experiment (some trends were observed in the photoinhibition-recovery experiment). There were however significant decreases in % DA Fv/Fm between inhibitor treatments of nitrogen limited *T. oceanica* and *Tetraselmis*. It is seems that the D1 protein repair cycle is active in the nitrogen limited samples. Perhaps this is due to the presence of intracellular nitrogen.

Photoinhibition-recovery experiment

There was a full recovery in relative PS II efficiency after high light exposure in *T. oceanica* when no lincomycin was present. The greatest recovery occurred in the GI 125 *T. oceanica*, due to the large initial drop in % DA Fv/Fm after high light exposure. The initial recovery present in the non-inhibitor treatment for *T. oceanica* was not present in the inhibitor treatment. There was less recovery seen in *Tetraselmis,* when compared to *T. oceanica*, and its % DA reached an average of 60%. The inhibitor affected *Tetraselmis* to a much lesser extent than *T. oceanica*. The recovery of PSII started between 0 – 20 minute time frame after high light exposure for *T. oceanica* and *Tetraselmis.* The data suggests that D1 protein synthesis is a quick response (<20 min.) mechanism which can repair photodamaged PSII systems. Also, this mechanism is more prevalent in *T. oceanica* and makes *T. oceanica* more resistant to high light exposure than *Tetraselmis.*

The initial drop (after high light, t=0 min.) in % DA Fv/Fm was greater with increasing GI's for nutrient replete (E) *T. oceanica* and *Tetraselmis* in the non-inhibitor treatment. The initial drop in % DA Fv/Fm in the nutrient replete inhibitor treatment was large for all GI's and comparable to the low % DA Fv/Fm value of the nutrient replete non-inhibitor GI 125 samples. It seems, as seen from the greater difference in % DA Fv/Fm right after high light exposure between inhibitor treatments in

lower GI's, that the D1 protein repair cycle is much more necessary in lower GI's.

The % DA Fv/Fm in the nitrogen limited non-inhibitor treatment right after high light exposure was significantly lower than the nutrient replete samples. Furthermore, the % DA Fv/Fm in the nitrogen limited non-inhibitor treatment right after high light exposure was similar to most samples in the inhibitor treatment. It could be that low nitrogen concentration can have a negative influence on the D1 protein repair cycle. It is also possible, as seen in previous studies (Klein 1988, Latasa 1995, Latasa and Berdalet 1994, Llewellyn and Gibb 2000, Ruivo *et al.* 2011), that there was an increased level of photo-protective xanthophyll pigments present during nitrogen limitation. This would also cause a decrease in the % DA Fv/Fm of nitrogen limited algae exposed to high irradiance.

Nutrient replete *T. oceanica* and *Tetraselmis* in the inhibitor treatment had on average 40% DA Fv/Fm recovery. This recovery is most likely due to the xanthophyll cycle. The low recovery of % DA Fv/Fm in the inhibitor treatment could be due to a low epoxidation rate of xanthophylls in the presence of photodamage. It could be that xanthophylls do not fully epoxidize as long is photodamage to the PSII apparatus is still present. Furthermore, it is possible for some D1 to still be present in lincomcyin treated samples, which would cause some recovery in % DA Fv/Fm [Alderkamp *et al.* 2013]. It is also unclear which percentage of the % DA Fv/Fm recovery in the non-inhibitor treatment is due to xanthophyll epoxidation, and which percentage is due to the D1 repair cycle. Pigment analyses (e.g. via high-pressure liquid chromatography) and immunochemistry blots for D1 protein are required for future PSII efficiency vs. irradiance experiments and photoinhibition-recovery experiments in order to separate the effect of xanthophyll and D1 protein repair cycle on the % DA Fv/Fm response.

Conclusion

First of all, using phytoplankton's minimum fluorescence proved to be an effective way to estimate the algae's increase in biomass and therefore the algae's growth phases.

Increasing growth irradiance had a positive effect on the phytoplankton growth rates. Nitrogen limitation stopped the biomass increase of algae.

The D1 protein repair cycle is an important mechanism to counteract photodamage to the PSII apparatus from excess irradiance. The D1 repair cycle was more abundant in *T. oceanica* (bacillariophyceae) than in *Tetraselmis* (chlorophyceae). It appears that *T. oceanica* is better equipped to deal with large variations in irradiance. Low irradiance acclimated algae seem to have an increased reliance on the D1 cycle after high light exposure, most likely due to having more active photosystems that can be damaged, a lower xanthophyll cycle activity and lower abundance of xanthophyll pigments. Vice versa, high irradiance acclimated algae have a lesser reliance on the D1 cycle. Lastly, nitrogen limitation had a negative effect on the photosynthetic efficiency of the algae. However, it is unclear if this is due to a lower D1 repair cycle activity or due to an increased level of photo-protective xanthophyll pigments.

References

Alderkamp A., Mills M., van Dijken G., Arrigo K. (2013) Photoacclimation and non-photochemical quenching under in situ irradiance in natural phytoplankton assemblages from the Amundsen Sea, Antarctica. *Mar Ecol Prog Ser* **475**: 15-34.

Aro E.M., Mccaffy S., Anderson J.M. (1994) Recovery from photoinhibition in peas (*pisum sativum L.*) acclimated to varying growth irradiances: Role of D1 protein turnover. *Plant Physiol.* **104**: 1033–1041.

Bachmann K.M., Ebbert V., Adams W.W., Verhoeven A.S., Logan B.A., Demmig-Adams B. (2004) Effects of lincomycin on PSII efficiency, nonphotochemical quenching, D1 protein and xanthophyll cycle during photoinhibition and recovery. *Funct Plant Biol* **31**:803–813.

Bouchard J.N., Roy S., Campbell D.A. (2006) UVB effects on the Photosystem Il - D1 protein of phytoplankton and natural phytoplankton communities. *Photochem. and Photobio*. **82**: 936-951.

Consalvey M., Perkins R.G., Paterson D.M., Underwood G.J.C. (2005): PAM Fluorescence: A beginners guide for benthic diatomists, *Diatom Research*, **20**: 1-22.

Demming-Adams B., Adams W.W. (1992) Photoprotection and other responses of plant to high light stress. *Annu Rev Plant Physiol Plant Mol Biol* **43**: 599–626.

Domingues N., Matos A.R., Marques da Silva J., Cartaxana P. (2012) Response of the diatom *Phaeodactylum tricornutumto* photooxidative stress resulting from high light exposure. *PLoS ONE* **7**(6).

Edelman M., Mattoo A.K. (2008) D1-protein dynamics in photosystem II: the lingering enigma. *Photosynth. Res*.**98**: 609–620.

Falkowski P.G., Laroche J. (1991) Acclimation to spectral irradiance in algae. *Journal of Phycology* **27**: 8–14.

Goss R., Jakob T. (2010) Regulation and function of xanthophyll cycle-dependent photoprotection in algae. Photosynth. Res. **106**:103–122

Guillard R.R., Ryther J.H. (1962) Studies on planktonic diatoms 1-*Cyclotella nana Hustedt* and *Detonula conferuacea* (Cleve) *Gran. Can. J. Microbiology* **8**: 229-239.

Guillard RR (1975) Culture of Phytoplankton for Feeding Marine Invertebrates. Culture of Marine Invertebrate Animals pp 29-60.

Hasle G.R. (1983) The marine planktonic diatoms *Thalassiosira oceanica sp.* nov. and *T partheneia*. J. Phycol **19**: 220-229.

Honeywill C., Paterson D., Hagerthey S. (2002) Determination of microphytobenthic biomass using pulse-amplitude modulated minimum fluorescence. *European Journal of Phycology*, **37**:4, 485-492.

Jansen M.A.K., Mattoo A.K., Edelman M. (1999) D1–D2 protein degradation in the chloroplast: complex light saturation kinetics. *Eur J Biochem* **260**: 527–532.

Jin E.S., Yokthongwattana K., Polle J.E.W. & Melis A. (2003*)* Role of the reversible xanthophyll cycle in the photosystem II damage and repair cycle in *Dunaliella salina. Plant Physiology* **132**, 352–364.

Kana T.M., Geider R.J., Crichley C. (1997) Regulation of photosynthetic pigments in micro-algae by multiple environmental factors: a dynamic balance hypothesis. *New Phytologist* **137**: 629–638.

Kirk JTO (1994) Light and photosynthesis in aquatic ecosystems, 2nd ed. Cambridge University Press.

Klein B. (1988) Variations of pigment content in two benthic diatoms during growth in batch cultures. *J. Exp. Mar. Biol. Ecol*. **115**: 237–248.

Kolber Z., Zehr J., Falkowski P. (1988) Effects of Growth Irradiance and Nitrogen Limitation on Photosynthetic Energy Conversion in Photosystem II. *Plant Physiol*. **88**: 923-929.

Latasa M. (1995) Pigment composition of *Heterocapsa* sp. and *Thalassiosira weissflogii* growing in batch cultures under different irradiances. *Sci. Mar*., **59**: 25–37.

Latasa M., Berdalet E. (1994) Effect of nitrogen or phosphorus starvation on pigment composition of cultured *Heterocapsa* sp. *J. Plankton Res*., **16**: 83–94.

Lavaud J., Rousseau B., Van Gorkum H.J., Etienne A. (2002) Influence of the diadinoxanthin pool size on photoprotection in the marine planktonic diatom *Phaeodactylum tricornutum*. *Plant Physiol* **129**: 1398−1406.

Llewellyn C.A., Gibb S.W. (2000) Intra-class variability in the carbon, pigment and biomineral content of prymnesiophytes and diatoms. *Mar. Ecol. Prog. Ser*., **193**: 33–44.

MacIntyre H.L., Kana T.M., Geider R.J. (2000) The effect of water motion on short-term rates of photosynthesis by marine phytoplankton. *Trends Plant Sci* **5**: 12–17.

Mattoo A.K., Hoffman-Falk H., Marder J.B., Edelman M. (1984) Regulation of protein metabolism: coupling of photosynthetic electron transport to *in vivo* degradation of the rapidly metabolized 32-kilodalton protein of chloroplast membranes. *Proc Nat Acad Sci USA* **81**: 1380–1384.

Muller P., Li X.P., Niyogi K. (2001) Non-photochemical quenching. A response to excess light energy. *Plant Physiol* **125**: 1558–1566.

Nishiyama Y., Allakhverdiev S.I., Murata N. (2006) A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochim Biophys Acta* **1757**: 742–749.

Nixon P.J., Barker M., Boehm M., de Vries R., Komenda J. (2005) FtsH-mediated repair of the photosystem II complex in response to light stress. *J Exp Bot* **56**: 357–363.

Park Y.I., Chow W.S., Anderson J.M. (1995) Light inactivation of functional photosystem II in leaves of peas grown in moderate light depends on photon exposure. *Planta* **196**: 401–411.

Provasoli-Guillard National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine USA.

Ruivo M, Amorim A, Cartaxana P (2011) Effects of growth phase and irradiance on phytoplankton pigment ratios: implications for chemotaxonomy in coastal waters. *J Plankton Res* **33**:1012–1022

Schubert H., Sagert S., Forster R.M. (2001) Evaluation of the different levels of variability in the underwater light field of a shallow estuary. Helgoland Marine Research **55**: 12–22.

Shelly K., Heraud P., Beardall J. (2002) Nitrogen limitation in *Dunaliella tertiolecta* (Chlorophyceae) leads to increased susceptibility to damage by ultraviolet-B radiation but also increased repair capacity. *Journal of Phycology* **38**: 713–720.

Six C., Finkel Z.V., Irwin A.J., Campbell D.A. (2007) Light variability illuminates niche-partitioning among marine picocyanobacteria. *PLoS ONE* **2**(12): e1341.

Tilman D., Kilham S., Kilham P. (1982) Phytoplankton community ecology: The role of limiting nutrients. *Annual Review of Ecology and Systematics* **13**: 349-372.

Tyystjarvi E, Aro EM (1996) The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity. *Proc Natl Acad Sci USA* **93**: 2213–2218.

Van de Poll W.H., Van Leeuwe M.A., Roggeveld J., Buma A.G.J. (2005) Nutrient limitation and high irradiance acclimation reduce PAR and UV-induced viability loss in the Antarctic diatom *Chaetoceros brevis* (Bacillariophyceae). *Journal of Phycology* **41**: 840–850.

Van de Poll W.H., Alderkamp A.C., Janknegt P.J., Roggeveld J., Buma A.G.J. (2006) Photoacclimation modulates excessive photosynthetically active and ultraviolet radiation effects in a temperate and an Antarctic marine diatom. *Limnol Oceanogr* **51**: 1239−1248.

Van de Poll W..H., Buma A.G.J., Visser R.J.W., Janknegt P.J., Villafane V.E., Helbling E.W. (2010) Xanthophyll cycle activity and photosynthesis of *Dunaliella tertiolecta* (Chlorophyceae) and *Thalassiosira weissflogii* (Bacillariophyceae) during fluctuating solar radiation. *Phycologia* **49**: 249–259.

Van de Poll W.H., Lagunas M., De Vries T., Visser R.J.W., Buma A.G.J (2011) Non-photochemical quenching of chlorophyll fluorescence and xanthophyll cycle responses after excess PAR and UVR in *Chaetoceros brevis*, *Phaeocystis antarctica* and coastal Antarctic phytoplankton. *Mar Ecol Prog Ser* **426**: 119−131.

Wilhelm C., Buchel C., Fisahn J., Goss R., Jakob T., Laroche J., Lavaud J., Lohr M., Riebesell U., Stehfest K., Valentin K, Kroth P. (2006) The regulation of carbon and nutrient assimilation in diatoms is significantly different from green algae. *Protist* **157**: 91–124.

Wu H., Roy S., Alami M., Green B.R., Campbell D.A. (2012) Photosystem II photoinactivation, repair, and protection in marine centric diatoms. *Plant Physiol* **160**:464–476.